

What is claimed is:

1. A method of delivering a protein to a domestic bird comprising administering to the bird in a whole-body spray an effective amount of a live avirulent derivative of an enteropathogenic bacterium comprising a recombinant gene that encodes for the expression of the protein, wherein the enteropathogenic bacterium is  
5 other than one that causes respiratory disease in birds.
2. The method according to claim 1 wherein the method comprises colonization by the enteropathogenic bacteria of the tissues of at least one of the gut associated lymphoid tissue (GALT), bronchus associated lymphoid tissue (BALT), lung, liver, spleen, bursa of Fabricius, and ceca of the domestic bird.
3. The method according to claim 2 where the colonization further comprises expression of the protein encoded by the recombinant gene.
4. The method according to claim 1 wherein the enteropathogenic bacterium is selected from the group consisting of *Escherichia*, *Klebsiella*, *Proteus*, *Yersinia*, and *Erwinia*, *Salmonella*, *Salmonella*-*Escherichia* hybrids, *Shigella*, *Campylobacter*, *Providencia*, *Morganella*, *Hafnia*, *Serratia*, *Edwardsiella*, *Enterobacter* and  
5 *Citrobacter*.
5. The method according to claim 4 wherein the enteropathogenic bacterium is selected from the group consisting of *Salmonella*, *Escherichia* and *Salmonella*-*Escherichia* hybrids.
6. The method according to claim 5 wherein the enteropathogenic bacterium is selected from the group consisting of *Escherichia coli*, *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella enteritidis*, *Salmonella dublin*, *Salmonella gallinarum*, *Salmonella pullorum*, *Salmonella arizona*, *Salmonella*  
5 *enteritidis*, *Salmonella heidelberg*, *Salmonella anatum*, *Salmonella hadar*, *Salmonella agona*, *Salmonella montevideo*, *Salmonella kentucky*, *Salmonella infantis*, *Salmonella schwarzengrund*, *Salmonella saintpaul*, *Salmonella brandenburg*, *Salmonella istanbul*, *Salmonella cubana*, *Salmonella bredeney*, *Salmonella braenderup*, *Salmonella livingstone*, *Salmonella berta*, *Salmonella california*, *Salmonella*  
10 *senftenberg*, *Salmonella mbandaka* and *Salmonella choleraesuis*.

7. The method according to claim 6 wherein the enteropathogenic bacterium is selected from the group consisting of *Salmonella typhimurium* strains  $\chi$ 3115 (ATCC 39961), and  $\chi$ 3137 (ATCC 39962).

8. The method according to claim 5 wherein the enteropathogenic bacteria are an avirulent *Salmonella* which contains a mutation in the *phoP* gene, wherein the avirulent *Salmonella* is unable to cause *Salmonella*-based disease symptoms and is able to colonize in lymphoid tissue for a sufficient time to induce antibody and cellular immunity, and wherein the strain retains the properties of avirulence and immunogenicity of a *Salmonella* strain selected from the group consisting of ATCC 53864, ATCC 53865, and ATCC 53866.

9. The method according to claim 1 wherein the enteropathogenic bacteria are a derivative of a pathogenic strain of bacteria characterized by:

a) a lack of a functioning native chromosomal gene encoding a first enzyme which is a  $\beta$ -aspartic semialdehyde dehydrogenase (Asd);

5 b) the presence of a first recombinant gene encoding a second Asd enzyme wherein the first recombinant gene cannot recombine to replace the defective chromosomal gene;

c) the presence of a second recombinant gene encoding a desired polypeptide; and

10 d) physical linkage between the first recombinant gene and the second recombinant gene, wherein loss of the first recombinant gene causes the bacteria to lyse when in an environment which requires expression of the first recombinant gene for cell survival.

10. The method according to claim 9 wherein the enteropathogenic bacteria are selected from the group of strains consisting of  $\chi$ 6097 (ATCC 67537),  $\chi$ 3520 (ATCC 53681),  $\chi$ 4072 (ATCC 67538),  $\chi$ 3008 (ATCC 53680),  $\chi$ 2108 (ATCC 53678), and  $\chi$ 6097 (ATCC 67813).

11. The method according to claim 5 wherein the enteropathogenic bacterium comprises a live avirulent *Salmonella*, having a mutation in a *cdt* gene, where the *Salmonella* has the phenotype of failure to colonize deep tissue of *Salmonella* deposit strain ATCC no. 55113.

12. The method according to claim 11 wherein the enteropathogenic bacterium is selected from the group of strains consisting of  $\chi$ 3958 (ATCC 55110),  $\chi$ 4323 (ATCC 55115),  $\chi$ 4346 (ATCC 55113),  $\chi$ 3940 (ATCC 55119), and  $\chi$ 4073 (ATCC 55118).

13. The method according to claim 6 wherein the enteropathogenic bacterium is a live avirulent *Salmonella choleraesuis* obtained from a pathogenic strain of *S. choleraesuis*, and where the avirulent *S. choleraesuis* has been made avirulent by an inactivating mutation in a *cya* gene and an inactivating mutation in a *crp* gene.

14. The method according to claim 13 wherein the enteropathogenic bacterium comprises strain  $\chi$ 3781 (ATCC 67923).

15. The method according to claim 6 wherein the enteropathogenic bacterium is a live avirulent *Salmonella typhi* that is obtained from a pathogenic *S. typhi* strain and is made avirulent by an inactivating mutation in the structural *cya* gene and an inactivating mutation in the structural *crp* gene.

16. The method according to claim 15 wherein the enteropathogenic bacteria are selected from the group of strains consisting of  $\chi$ 3927 (ATCC 55117) and  $\chi$ 4323 (ATCC 55115).

17. The method according to claim 4 wherein the live avirulent derivative of an enteropathogenic bacterium is a *Salmonella* and the protein comprises an O-antigen of an avian pathogenic gram negative microbe (AP<sub>G-N</sub>), where the O-antigen is encoded by an *rfb/rfc* gene cluster of the AP<sub>G-N</sub> microbe that is stably integrated into the *Salmonella* chromosome and having a mutation in the *Salmonella rfb* gene cluster or in the *Salmonella rfc* gene which inactivates expression of *Salmonella* O-antigen, wherein the recombinant *Salmonella* strain is an attenuated mutant of a virulent *Salmonella* strain.

18. The method according to claim 17 wherein the O-antigen comprises at least one *E. coli* specific antigen selected from the group consisting of LPS O-antigen of strains O1, O2, O35 and O78.

19. The method according to claim 4 wherein the protein is selected from the group consisting of immunoregulatory peptides, immunoregulatory proteins and growth factors.

20. The method according to claim 19 wherein the immunoregulatory peptide or protein is selected from the group consisting of macrophage colony stimulating factors, granulocyte colony stimulating factors, mixed colony stimulating factors, macrophage chemotoxins, macrophage inhibition factors, leukocytes, inhibitory factors, lymphotoxins, blastogenic factors, interferon, and interleukins.
21. The method according to claim 19 wherein the protein is a growth factor.
22. The method according to claim 1 wherein the spray administration comprises spraying droplets having diameters in the range of from 50 microns to 150 microns.
23. The method according to claim 22 wherein the spray is administered in a dose of from about  $10^4$  to about  $10^8$  colony forming units of the live avirulent derivative of a pathogenic bacterium.
24. The method according to claim 23 wherein the spray is administered in a dose of from about  $10^5$  to about  $10^7$  colony forming units of the live avirulent derivative of a pathogenic bacterium.
25. The method according to claim 24 wherein the spray is administered in a dose of at least about  $1 \times 10^6$  colony forming units of the live avirulent derivative of a pathogenic bacterium.
26. The method according to claim 23 wherein the poultry are less than 104 weeks of age.
27. The method according to claim 26 wherein the poultry are 3 weeks of age or less.
28. The method according to claim 27 wherein the poultry are less than one day of age.
29. The method according to claim 26 wherein the poultry are chickens.
30. The method according to claim 26 wherein the spray administration is followed by administration of at least one booster dose of the vaccine.
31. The method according to claim 30 wherein the booster dose of vaccine is administered by spray administration.
32. The method according to claim 30 wherein the booster dose of vaccine is administered in drinking water.

33. The method according to claim 30 wherein a booster dose is administered 14 days after the spray administration.

34. A domestic bird that has been treated by the method of claim 1.